The ROCK inhibitor Y-27632 improves recovery of human embryonic stem cells after fluorescence-activated cell sorting with multiple cell surface markers.

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Public Summary:
Due to the inherent sensitivity of human embryonic stem cells (hESCs) to manipulations, the recovery and survival of hESCs after fluorescence-activated cell sorting (FACS) can be low. Previous studies have demonstrated that the p160-Rho-associated coiled kinase (ROCK) inhibitor, Y-27632, enhances survival of hESCs upon single-cell dissociation, as well as enhancing recovery from cryopreservation. We therefore examined the application of Y-27632 to hESCs after FACS to improve survival in both feeder-dependent and feeder-independent growth conditions. We demonstrate that the application of Y-27632 to hESCs after cell sorting improves cell recovery with no observed effect on pluripotency, and enables the consistent recovery of hESCs by FACS using multiple surface markers. This improved methodology for cell sorting of hESCs will aid many applications such as removal of hESCs from secondary cell types, identification and isolation of stem cell subpopulations, and generation of single cell clones.

Scientific Abstract:
BACKGROUND: Due to the inherent sensitivity of human embryonic stem cells (hESCs) to manipulations, the recovery and survival of hESCs after fluorescence-activated cell sorting (FACS) can be low. Additionally, a well characterized and robust methodology for performing FACS on hESCs using multiple-cell surface markers has not been described. The p160-Rho-associated coiled kinase (ROCK) inhibitor, Y-27632, previously has been identified as enhancing survival of hESCs upon single-cell dissociation, as well as enhancing recovery from cryopreservation. Here we examined the application of Y-27632 to hESCs after FACS to improve survival in both feeder-dependent and feeder-independent growth conditions. METHODOLOGY/PRINCIPAL FINDINGS: HESCs were sorted using markers for SSEA-3, TRA-1-81, and SSEA-1. Cells were plated after sorting for 24 hours in either the presence or the absence of Y-27632. In both feeder-dependent and feeder-independent conditions, cell survival was greater when Y-27632 was applied to the hESCs after sort. Specifically, treatment of cells with Y-27632 improved post-sort recovery up to four fold. To determine the long-term effects of sorting with and without the application of Y-27632, hESCs were further analyzed. Specifically, hESCs sorted with and without the addition of Y-27632 retained normal morphology, expressed hESC-specific markers as measured by immunocytochemistry and flow cytometry, and maintained a stable karyotype. In addition, the hESCs could differentiate into three germ layers in vitro and in vivo in both feeder-dependent and feeder-independent growth conditions. CONCLUSIONS/SIGNIFICANCE: The application of Y-27632 to hESCs after cell sorting improves cell recovery with no observed effect on pluripotency, and enables the consistent recovery of hESCs by FACS using multiple surface markers. This improved methodology for cell sorting of hESCs will aid many applications such as removal of hESCs from secondary cell types, identification and isolation of stem cell subpopulations, and generation of single cell clones. Finally, these results demonstrate an additional application of ROCK inhibition to hESC research.